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TRANSLATION NO. 115

DATE: Sept 1968

DEPARTMENT OF THE ARMY
Fort Detrick
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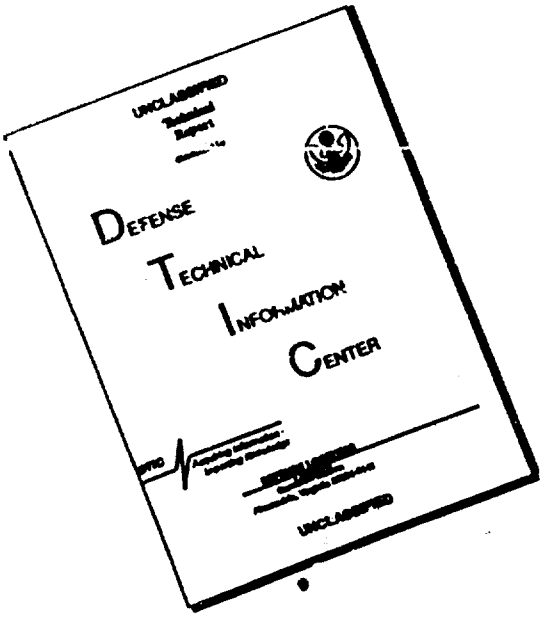

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Journal of Microbiology, Epidemiology and Immunobiology, USSR, No. 1-2, 1943, 87-90

Experimental Material on the Etiology of the Autumnal Form of Encephalitis, Report II

by A. K. Shubladze

While studying the autumnal form of encephalitis, the second expedition of the N. K. Zdrava USSR isolated 48 strains of the virus from patients and deaths of this encephalitis.

In the third expedition of the N. K. Zdrava USSR these data were confirmed by us with the isolation of two more strains; one from a patient's blood and one from the brain of a corpse.

The strains of the third expedition were identical with those strains of the second expedition. As the first communication read, biologically and serologically the virus, obtained by us, is identical with the virus of Japanese (summer) encephalitis.

In the second expedition, and by further work of the third, it was established that the virus of autumnal encephalitis was present in mosquitoes of the infected areas. Spontaneously infectable were the Culex pipiens and Culex tritaeniorhynchus.

These data are confirmed by the finding of spontaneously infected mosquitoes in Japan during summer encephalitis. There was established, by the works of Mitamura and co-workers, that during Japanese encephalitis, besides the detection of a spontaneous susceptibility in the mosquitoes, it is possible to detect the ability to carry this virus in well people and animals living in the centers of infection, and the virus-bearability leads to the accumulation of antibodies in the blood.

In the epidemic of 1938 in Tokyo, the virus of the encephalitis was

found in well people, in 6 cases of 82, and among numerous well animals; in 4 of 90 rats, in 1 of 3 horses, and in 2 of 126 examined sparrows. Kill observed viremia in 1 horse, a week after it arrived in Tokyo in the epidemical season, and later the development of antibodies in the blood. Mitamura and co-workers explain the presence of a high neutralising activeness in the serum of horses of the epidemical region (Tokyo, Okiyam and others) by the expansive dissemination of the virus-bearability. Tests by the author established the following facts on the presence of a high titer of antibodies to the virus of encephalitis, in the Tokyo area and surrounding areas; antibodies were observed in 86% of 242 people, in 98% of 50 horses, in 86% of 42 cows, and in 17% of 85 examined pigs.

Taking this data into account, relative to Japanese encephalitis, as being epidemiologically important, we conducted studies on the blood of well people and birds, located in the region infected with the autumnal form of encephalitis, on virus-bearability.

Blood was obtained from 315 people. Fresh, uncoagulated blood of 3-5 people was mixed in equal volumes and introduced intracerebrally in doses of 0.03 cm^3 to three healthy mice. There were examined 68 portions of mixed blood in this way. After 9 days the mice were killed and their brains were used on passages on fresh mice. Two successive passages were made.

From 2 portions of blood, mice on the second passage became infected with an experimental encephalitis. The first portion of blood was composed of a mixture from 3 people and the second from 5.

After a 3 month storage of these strains in 50% glycerine, it was possible to restore and study only one strain of the isolated virus. The obtained virus caused infection in mice upon intracerebral, intraperitoneal,

intranasal and subcutaneous injection and filtrated easily through a Berkefield-Shamberlak candle. Tests of neutralization of the strain with specific serums of recovered patients and immune rabbit serum indicated, that the virus, isolated from healthy people, conducts itself in these tests just as the stra ns isolated from patients.

Next we studied 94 birds, caught in the center of infection, in regard to the blood and brain.

The brain and blood of 1-5 examples of the same type of bird were mixed, made into an emulsion, and injected intracerebrally in doses of 0.03 cm^3 into 3 mice, the brains of whbh were passed after 9 days on fresh mice. In one test, mice infected with blood and brain of 5 birds (sparrows) became infected with encephalitis. After 3 months storage, in glycerine, this obtained strain was studied and identified with strains of human origin. Data on these tests are in Table 1.

Thus, the virus-transmissibility of the birds in the center of infection was established.

The detection, by the Japanese authors, of the virus-transmissibility in mosquitoes and sparrows and the isolation of a strain of the encephalitis virus by us, gives basis to surmise the presence of birds in the regions of infection spontaneously infected with encephalitis. Taking into consideration that mosquitoes feed on birds, that certain birds of the sparrow group are susceptible to summer encephalitis, causes us to consider the bird as a possible reservoir of the virus of autumnal encephalitis.

The second phase of our work was studying the blood of healthy people and animals, living in the area of infection, for the presence of antibodies to encephalitis. Blood was obtained from 315 people and 150 horses. All the

animals, from which blood was obtained, had lived in the infected area more than 2 years.

Here are the results of tests of the neutralisation of virus of autumnal encephalitis with 100 serums of healthy people and 100 serums of horses.

The virus strain Zag., used for passages, was used in an emulsion at 10-5 and 10-6. The last dilution of the strain, which still caused infection and death of mice upon intracerebral injection, equaled 10-7; this strain was used in a mixture with serum of blood selected from horses outside the area of infection as a control.

Each of these viruses was mixed with an equal quantity of undiluted serum (volume 0.15 cm^3); after 2 hours in a thermostat at 37° the mixture was injected intracerebrally to three mice.

As Table 2 indicates, of 100 serums selected from humans, 11 possess virus neutralising properties, and in 4 of them these properties were clearly marked, the virus was neutralised fully, as in the dilution 10-6, so in 10-7. Three serums of patients recovered from autumnal encephalitis neutralised the virus in an identical manner, just as did the 4 serums of healthy people, while 5 serums, selected from humans living outside the infectious area, did not protect the mice from the virus in the said dilutions (even in the dilution 10-7). Of the 100 horse serums, 35 neutralised the virus and 17 of them neutralised it in the 10-5 dilution. Five serums of horses living outside the center of infection gave no neutralisation.

We used only one serum of a horse, immunised to tick encephalitis. This serum, taken as a control, fully neutralised the virus of autumnal encephalitis.

However, 11% of the human serums and 35% of the horse serums, taken in the infected area, indicated the presence of antibodies, capable of neutralizing virus of autumnal encephalitis.

Thus the autumnal form of encephalitis, present in the USSR, is similar to the summer (Japanese Type B) encephalitis, not only according to agent properties, but also according to epidemiological features.

The low percentage (in comparison with those of Japanese authors) of positive serums in our studies (11% of human and 35% of horse) points to a smaller distribution of this virus in our local. The low rate of virus transmissibility in people and animals in the infected area also points to this.

Table I. Analysis of the brain and blood of birds for the content of Virus of encephalitis.

Type of bird	Number of birds	Results of analysis
Sparrow	38	In one case virus was obtained from 5 sparrows
Pheasant	16	Mice healthy
Duck	13	Infection of mice with brain and blood of one duck. Bacteriological contamination.
Wagtail	11	Mice Healthy
Swallow(Martin)	6	dito
Pigeon	3	Mice infected. Bacteriological contamination.
Magpie	1	Mice healthy
Seagull	1	"
(Sky)lark	1	"
Woodpecker	1	Mice infected. Bacteriological contamination.
Owl	1	" "
Quail	1	Mice healthy
Cuckoo	1	"
Total	94	.....

TEXT: Experimental material on the etiology of the autumnal form of encephalitis.

Table II. Tests of neutralization with serums of healthy people and horses.

Serum	Positive neutralization with a dilution of the Zag. strain.....		General quantity of serums	General quantity of positive neutralizations....
	1:100 000	1:1 000 000	Figures and not percentage)	
Healthy people	4	7	100	11
Healthy people; living outside the region of infection	0	0	5	0
Recovered patients	3	—	3	3
Horse	17	18	100	35
Horse; not living in the infectious center..	0	0	5	0
Horse; one horse immunized to tick encephalitis..	1	—	1	1

TEXT: Experimental material on the etiology of the autumnal form of encephalitis.

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NO. 8 - 1940 Pages 29-35

Journal of Microbiology, Epidemiology and Immunobiology No. 1-2, 1943, Pages 87-90
(Summary translated) USSR

Experimental Data on the Etiology of Autumnal Form of Encephalitis (2nd report), by A. K. Shubladse

1st Report

Conclusions

To date of report 48 strains of virus have been isolated from patients and people who died from the disease.

These strains and all strains tested in all experiments are identical (biologically and serologically) to Japanese encephalitis.

Vectors were ascertained to be the Culex pipiens and Culex tritaeniorhynchus. This coincides with reports from Japan on the vectors of encephalitis.

Humans and animals were also named as vectors of the virus, ailing or healthy.

Reports of the epidemic in Tokyo in 1938 stated (according to this author) that humans and horses had a high titer of antibodies of encephalitis, and figures are 86% in humans, 98% in horses, 86% in cows and 87% in pigs.

Tests on humans and birds, not infected or having been infected, were made to determine their virus capacity at the time of this epidemic; blood from humans was blended (three blood samples) and injected into mice (white). An experimental encephalitis developed. The strain was identical to that of infected humans.

Only one strain survived a 3 month storage in 50% glycerine. Strains were easy to handle and filtered nicely.